Human gene therapy

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Backgrounder

HUMAN GENE THERAPY

THOMAS CURREN
SCIENCE AND TECHNOLOGY DIVISION

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TABLE OF CONTENTS

Page

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HUMAN GENE THERAPY

INTRODUCTION

Humankind is subject to a great variety of diseases, many caused by infectious agents such as bacteria and viruses, and others whose causes have not been determined. A third group of diseases includes those caused by a defective gene, or genes. In genetic disease, a portion of an individual's inherited genetic complement is abnormal and produces an observable symptom, or symptoms, denoting a diseased condition. Such genetic diseases range in effect from the trivial to the extremely serious. In the most severe cases, gross disability and/or premature death are the characteristic manifestations.

It is estimated that 1-2% of newborn infants suffer from some specific genetic defect.(1) In recent years, the extension of knowledge of human genetics and molecular biology has allowed scientists to identify some of the specific genes which are responsible for inherited diseases. With the ability to identify disease-causing genes has come the potential to correct the molecular defect which caused the disease in the first place.

Medical science has been able for some time to treat successfully certain genetic diseases, including juvenile-onset diabetes and some physical defects, through the use of therapeutic chemicals, diet, or surgical techniques. Gene therapy, however, involves medical intervention of a qualitatively different type wherein the actual cause of the disorder is identified, targeted and then corrected at the molecular level.

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(1) Office of Technology Assessment (OTA), Human Gene Therapy: Background Paper, Congress of the United States, Washington, D.C., 1984, p. 1.

For the purposes of this paper, "human gene therapy" will be defined as follows:

> the deliberate administration of genetic material into a human patient with the intent of correcting a specific defect.(2)

The type of genetic defects discussed in this paper are those typically recognized as diseased conditions. Such general characteristics as intelligence, behaviour and physical appearance, traits which are governed by a multiplicity of genes, and which, moreover, are usually assessed in highly subjective terms, will be specifically excluded from this discussion.

Gene therapy techniques can be segregated into two categories: somatic cell gene therapy and germ line gene therapy.

In the first category are those prospective techniques which seek to correct a genetic defect through the modification of somatic, or non-reproductive, cells; such therapeutic intervention, although it will cause a permanent and reproducible change in the individual, will not affect the reproductive cells and will not, therefore, be passed on to future generations.

In contrast, germ line gene therapy would seek to correct a genetic defect in such a manner that not only would the treated individual be cured, but the change would be inherited by the individual's progeny. In this case, the therapeutic intervention would effect a small but permanent alteration in the patient's genetic make-up and, by extension, in the human gene pool.

The issue of human gene therapy is, for many people, highly charged, partly because it is technically a radically new step in medical practice and partly because the recombinant DNA technology that underwrites the proposed therapy is very complex and sometimes controversial. Also, there is a feeling among segments of the general public that the genetic manipulation of humans is simply not an acceptable activity.

There are a number of other issues associated with gene therapy which are also pertinent to the present discussion. One is

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(2) Ibid., p. 2.

controversial in itself: the practice of prenatal screening to identify genetic defects, or other abnormalities, in the fetus. Although there is a small risk of accidental miscarriage associated with some of the screening techniques, the principal concern centres on the possibility of a woman's choosing to have an abortion in the event that the fetus is diagnosed as having a major defect, genetic or otherwise. For many people in Canadian society, this creates a serious moral dilemma.

There have been suggestions, though very limited clinical evidence, that some genetic defects or characteristics may predispose individuals to environmental illnesses through a greater sensitivity to chemical contaminants or ionizing radiation. Such suggestions nave implications for those who work in certain occupational situations where a quantifiable level of exposure to hazardous substances is an inescapable reality.

Gene therapy is based on complex technology and on continuing research at the frontiers of medical and biological science. Inevitably, the technology has given rise to concerns about certain social and ethical issues that are, or will be, involved. This paper provides an introduction to the technology and the science upon which it is based. Some of the important social and ethical questions are identified and briefly discussed.

An effort has been made to frame the discussion in language that the non-scientist will understand, but it is inevitable that some complex technical concepts and terminology have been included. An extensive glossary of terms is appended to the paper, as well as a list of selected references which will amplify many of the important points.

GENETICS AND INHERITANCE

All living creatures are composed of cells, the basic integrated units of biological activity. All living organisms are capable of reproduction, ensuring the perpetuation of the species through the birth of successive generations. The organic thread that systematically binds each succeeding generation to its parent through shared physical

characteristics is deoxyribonucleic acid, DNA, a complex chemical substance in the nucleus of a cell which contains the hereditary information in all but the most primitive organisms.

In humans, as in all highly-developed organisms, the DNA is contained in the cell nucleus in discrete structures known as chromosomes. Human cells have 23 pairs of chromosomes, for a total of 46. All of the essential genetic information for the life and reproduction of the human species is contained in the chromosomes. These microscopic structures, then, constitute the human genome.

Most higher organisms, including humans, contain chromosomes in pairs, a condition known technically as the diploid state. Reproductive cells, the sperm and egg cells, contain only a single set of 23 chromosomes, a state known as haploid. In the process of reproduction, when the sperm and egg cells unite through fertilization, the two haploid cells form a diploid zygote which, through cell division, eventually produces an embryo and, ultimately, a newborn child. The gender of the resultant individual is determined by the sex chromosomes, one each from the egg and the sperm cell. Each egg cell will have one X chromosome; each sperm cell will have either an X or a Y chromosome. A pair of X chromosomes will produce a female offspring and an X-Y combination will produce a male. Thus, the sex of the offspring will be determined by the male parent. A significant fact about the human genome is that the X chromosome is much larger than the Y. Therefore, visual study of the chromosomes in the diploid cell will reveal the sex of the individual. Sex-typing can be done with fetal cells in a standard prenatal diagnostic procedure.

A normal human cell has one pair of sex chromosomes and 22 pairs of non-sex chromosomes, known as autosomes. The individual units of inheritance, the genes, are contained in the chromosomes. The subject of genetics and inheritance is complex, but for the purposes of this paper some basic facts will be sufficient for an appreciation of the significant points and will prepare the reader for a discussion of genetic disease and gene therapy.

Most people are familiar with the fact that the basic laws of inheritance were originally discovered by a 19th century Austrian monk

and botanist named Gregor Mendel. Mendel's classic studies of the inheritance of certain traits in peas opened the door to our present understanding of genetics. Some traits, such as flower colour in the pea plants studied by Mendel, are governed by single genes and are known as single gene traits. With such traits, the pattern of inheritance is usually simple and therefore easily studied.

Many traits, however, are determined by the interactions of several genes. These multigene traits do not follow the simple Mendelian patterns of inheritance and are typically difficult to study. Many common human characteristics, including eye colour, height and intelligence, for example, are multigenic or polygenic.

Because chromosomes are present in pairs, the genes that govern the various human traits and characteristics also exist in pairs. (An obvious exception to this rule occurs in the male where the two sex chromosomes, the X and Y, are different and complementarity is lacking for the X chromosome. This is discussed below.) The paired nature of genes produces a complication in inheritance because genes may be either dominant or recessive.

A recessive gene will only be expressed if it is paired with a similar recessive gene, a condition known as the double recessive. If a recessive gene is paired with a dominant gene, the effect of the former will be masked, either completely or in part. Where the two genes in a pair are identical, for example in the double recessive condition, the individual is said to be homozygous for that gene; where the genes are different, the term heterozygous is used.

Certain traits (and diseases, also) are governed by genes on the X chromosome. This is significant for males because they possess only one X chromosome (contributed by the mother) and lack at least part of the balancing set of genes that females have by virtue of their possession of a second X chromosome. There are few specific traits, other than the obvious one of sex determination, and no known diseases, carried in genes on the Y chromosome.

It is necessary also to recognize that an individual's overall characteristics and performance are governed by a complex

interaction of genetic inheritance and environmental influences. Height is a good example. Maximum potential height is governed by the genes, but the expression of the genetic potential is profoundly influenced by a number of factors, especially the nutritional status of the individual during the growing years. Basic intelligence is also governed by inheritance, but the level of expressed intelligence in any individual is, to a large degree, determined by such environmental factors as early nutritional and health status, environment, and education.

The total genetic complement of the human being is the product of complex evolutionary processes which occurred over the millennia. The "ideal" human organism would contain a perfectly balanced and integrated genome, with each gene functioning normally to produce a totally healthy individual. Such an ideal individual does not exist because the genetic material, DNA, is subject to random changes in its molecular structure. These changes are called mutations and, although small, they are usually deleterious, and sometimes significantly so, causing some abnormality in function or structure in the affected individual. When the mutation is significant enough to cause a discernible metabolic or physical defect, as might happen, for example, if an abnormal enzyme were produced by the body, the defect would be classed as a genetic disease.(3)

GENETIC DISEASES

The idea that some human diseases might be genetic in nature and follow a definite pattern of inheritance was first advanced by a British physician and scientist, Archibald Garrod, around the turn of the century.(4) Garrod suggested that some human diseases might be due to

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(3) An enzyme is a complex protein produced by living cells and which catalyzes specific biochemical reactions in the body.

(4) OTA (1984), p. 13.

"inborn errors of metabolism" and, further, that such biochemical defects could be caused by genetic abnormalities whose inheritance pattern might follow the Mendelian principles.

Garrod's postulation has proved to be correct and a number of genetic diseases have been shown to be due to abnormal proteins whose synthesis in the body is governed by similarly abnormal genes. The first biochemical or molecular disease described was a blood disease, sickle cell anemia, in which an abnormal hemoglobin protein was found to be the causal factor.(5)

There are almost four thousand single-gene, or monogenic, human genetic diseases;(6) fortunately, most are extremely rare. Single-gene diseases are those in which the disease syndrome is caused by one abnormal gene. In this group, there are both dominant and recessive diseases. In the former example, the disease will be expressed when only one of the two chromosomes in a pair carries the abnormal gene. In a recessive disease, both chromosomes must carry the abnormal gene for the disease to be present. More than 500 genetic diseases are single-gene recessive disorders.

Some genetic diseases are described as sex-linked because the abnormal gene is located on the X chromosome. Many genetic diseases are multigenic or polygenic; that is, a number of interacting genes cause the disease. Examples of the various types of diseases are described below.

A. Single Gene Diseases

ADA Deficiency: ADA stands for adenosine deaminase, an enzyme whose absence from the body leads to metabolic errors that in turn cause inhibition of the immune system. The disease is an autosomal dominant disorder; that is, the gene is located on an autosome (non-sex

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(5) Linus Pauling et al, "Sickle Cell Anemia: A Molecular Disease," Science, Vol. 110: 543-548, 1949.

(6) Eve K. Nichols, Human Gene Therapy, Harvard University Press, 1988, p. 17.

chromosome) and only one chromosome in the pair need carry the defective gene for the disease to be expressed. ADA deficiency is manifested at birth and the affected children die within two years without treatment. This is a rare disease; there are 40 to 50 cases reported worldwide each year.

Huntington Disease: Also known as Huntington chorea, this disease does not become evident until the victim is between 30 and 50 years of age. The disease is invariably fatal and death is caused by progressive deterioration of brain tissue. Symptoms of the disease include dementia and disorders of movement. The disease is an autosomal dominant, and occurs in an estimated one in 10,000 live births.

Polycystic Kidney Disease: A dominant disorder, polycystic kidney disease involves a progressive deterioration of kidney function which is associated with the development of large numbers of cysts within the kidneys.

Cystic Fibrosis: An autosomal recessive disorder, CF is the most common life-threatening genetic disease of children, affecting about one in every 1,800 births in Canada. The specific cause of CF is not known and the disease is incurable. Treatment methods have improved in recent years but most victims die before age 30. CF is characterized by production of thick, glue-like mucus which causes respiratory and digestive problems. Death is caused by mucus in the lungs and a deficiency in pancreatic function.

PNP Deficiency: PNP stands for the enzyme purine nucleoside phosphorylase. The absence of this enzyme results in metabolic disorders which cause a breakdown in the immune system in a manner somewhat similar to ADA deficiency (described above). PNP deficiency is an autosomal recessive disorder which is extremely rare; only about 9 cases are reported each year.

Tay Sachs Disease: An autosomal recessive disorder, this disease affects newborn children and causes retardation of development, paralysis, dementia and blindness. Death is inevitable, usually occurring before the child reaches the age of four. The defective gene governs the production of an enzyme, hexosaminidase A, which is involved in the metabolism of brain tissue. The defective gene has both an ethnic and geographical link, being found in highest frequency among Ashkenazic Jews of Eastern European origin. In the United States, the number of cases per year has dropped to about 10 as a result of a successful carrier-screening program.

B. Sex-Linked Diseases

Muscular Dystrophy (Duchenne Type): This is an X-linked disorder caused by a recessive gene. It therefore mostly affects males. Females may carry the defective gene on one X-chromosome but the effects would be masked if there were a normal gene on the second X-chromosome. The disease affects muscle metabolism and usually causes death by age 20. The disease is found in one in about 7,000 live births.

Hemophilia: This so-called "bleeders" disease was once fairly common among European royal families where marriages between closely related individuals were frequent. The disorder is caused by recessive genes which are X-linked. The disease is most often seen in males and is typically transmitted by females who are carriers of the genes, but are themselves without symptoms. Hemophilia occurs in about one in 10,000 live births.

Lesch-Nyhan Syndrome: This is an X-linked disease which is caused by a deficiency in the enzyme, hypoxanthine-guanine phosphoribosyl transferase (HPRT). The disease is particularly tragic in that children are affected and the syndrome is characterized by compulsive self-mutilation and other mental and behavioural disorders. A rare disease, Lesch-Nyhan syndrome occurs in one in 100,000 live births.

C. Chromosome Disorders

Some serious genetic diseases are the result of abnormal numbers of chromosomes in the cells. This group includes disorders in both the autosomes and sex chromosomes.

Down Syndrome: Once called "mongolism", Down syndrome is caused by an extra copy of chromosome number 21, a condition known as "trisomy". The most serious symptoms are mental retardation, congenital heart defects, increased susceptibility to infection, and shortened life span.

Klinefelter Syndrome: A disorder in males caused by one or more extra X chromosomes, most often the XXY configuration. Symptoms include infertility, small testes, poorly developed secondary sex characteristics, and, in many victims, subnormal intelligence.

Turner Syndrome: A disorder in females usually caused by the lack of one X chromosome. Symptoms include short stature, webbing of the neck and failure of secondary sexual development. Intelligence is normal.

D. Multigenic Diseases

Included in this category are a number of diseases which are not only caused by defective genes but may also be influenced by environmental factors, including diet and such habits as smoking. Atherosclerosis, for example, is the most common form of arteriosclerosis ("hardening of the arteries") and is characterized by localized deposits of fatty material (lipids) in the walls or the chambers of blood vessels. The disease can be the result of defects in lipid (fat) metabolism, many of which have a genetic basis. Also, some human cancers and mental illnesses are suspected of being caused by a number of genes acting in concert. Environmental factors are possibly important in these diseases, also.

MOLECULAR GENETICS: DNA AND GENES

In 1953, an American geneticist, James Watson, and Francis Crick, a British biophysicist, working together at Cambridge University, postulated the molecular structure of DNA. Building upon decades of research into the molecular basis of genetics and inheritance, their discovery of the structure of DNA set the stage for the revolutionary advances in knowledge and technology that today permit scientists to manipulate the basic chemistry of life. While the principles of heredity had been known since the rediscovery of Mendel's studies early in this century, the mechanism by which genetic information is passed from one generation to another had remained a mystery.

Deoxyribonucleic acid, DNA, is a complex double-chained molecule twisted into a helical form. The DNA molecule, in popular schematic representation, resembles a spiral ladder in which the sugar-phosphate "sides" are linked together by "rungs" which are, in fact, pairs of chemicals known as bases. There are only four bases in human DNA: adenine, thymine, guanine, and cytosine. In the DNA molecule, adenine is always paired with thymine, and guanine is always paired with cytosine: in biochemical shorthand, the base-pairs are represented as A-T and G-C.

In simple terms, the four bases represent the genetic alphabet and the sequence of the base-pairs along the length of the DNA molecule comprises a biochemical vocabulary which encodes the genetic information essential to all life processes. Also, the absolute specificity of the base-pairing, A-T and G-C, provides a mechanism by which each parent DNA molecule can be copied to form two daughter DNA molecules. The process is known as replication and is possible because the two sides of the DNA molecule are not identical, but complementary.

When the DNA molecule splits in the process of cellular reproduction, it in a sense "unzips" along its length, separating the base-pairs. Each half of the DNA molecule then serves as a template for replication; the cell synthesizes new bases to form the "rungs" of the DNA ladder, and new sugar-phosphate molecules to form the "sides" of the

structure. The specificity of the base-pairing ensures that, when the two daughter DNA molecules are formed from the two templates of the original molecule, each will be identical to the parent and to each other.

A gene may be defined in a molecular sense by describing specifically how it regulates metabolic activity. Early in the evolution of knowledge of molecular genetics, it was hypothesized that a gene directed the production of an enzyme. This was the "one gene: one enzyme" hypothesis. However, because genes also governed the production of proteins that were not enzymes, the hypothesis was modified to "one gene: one protein".

Later, it was found that the production of some complex proteins was governed by more than one gene. For example, the protein hemoglobin, which is responsible for the oxygen-carrying capacity of the blood, consists of two polypeptide chains, each of which is coded by a separate gene.(7) Thus, in current thinking, a gene is regarded as a sequence of DNA which codes for a specific polypeptide.

An average-sized human chromosome contains on the order of 100 million base-pairs. The genes, which are contained in the chromosomes, consist of specific sequences of these base-pairs. The number of base-pairs in a particular gene will correlate with the size and complexity of the particular polypeptide whose production it governs. Each amino acid in a polypeptide is coded by three bases, or nucleotides, in the DNA of the gene. For each of the 20 amino acids found in humans, these "triplet codes" have been identified. A "typical" gene which codes for a polypeptide, or a protein, might consist of several thousand base-pairs.

Scientists are now able to determine the sequence of bases in the DNA molecule and in so doing have developed an understanding of the genetic vocabulary. This has been accomplished through a procedure known as sequencing. The technique is very complex and involves the use of enzymes extracted from bacteria which slice the intact DNA molecule into fragments at particular nucleotide sequences. These fragments are

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(7) A polypeptide is a large molecule composed of a chain of amino acids. It is similar to a protein, but smaller. As indicated, a protein may consist of several polypeptides.

separated and further sub-divided by chemical methods. The specific base-pair sequences of the fragments are then deduced from the accumulated information.(8)

The ability to identify genes at the molecular level as specific chemical entities has allowed scientists to isolate individual genes and, moreover, has led to the ability to produce multiple copies of a gene through a process known as gene cloning. In simple terms, gene cloning involves isolating a gene and then inserting it into a cloning vehicle: the cloning vehicle may be a bacterium, or a bacterial virus (bacteriophage). The isolated gene is inserted or "spliced" into the DNA of the bacterium or virus using restriction enzymes. The microorganism is then allowed to multiply through its natural growth cycle, reproducing the inserted gene in the process.

Since microorganisms reproduce very rapidly, billions of copies of the cloned gene may be produced within a day or less. For example, many bacteria divide every 40 minutes or so. From a single bacterial cell dividing at that rate, almost 70 billion cells could be produced within 24 hours, assuming ideal growth conditions. Viruses will reproduce with similarly impressive speed.

Scientists have now isolated and cloned some 300 human genes, including normal genetic counterparts of the defective genes that cause a number of genetic diseases. Included on this list are ADA deficiency, PNP deficiency, Lesch-Nyhan syndrome, thalassemia, and hemophilias A and B.

At first glance, it would seem that the possession of a cloned normal gene, together with proven recombinant DNA technology, places medical science on the threshold of effective gene therapy. Indeed, only a few years ago it was believed that such therapy was imminent. Unfortunately, the effective insertion of normal genes into mammals has proven to be more difficult than anticipated. In the next section, the proposed techniques of gene therapy will be discussed.

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(8) The bacterial enzymes used to fragment the DNA molecule are known as restriction endonucleases. These same enzymes are used in recombinant DNA technology, a technology popularly known as "gene splicing."

SOMATIC CELL GENE THERAPY

It was stated earlier in this paper that there are two basic categories of gene therapy: namely, somatic cell gene therapy and germ line gene therapy. Technologically, germ line gene therapy is by far the more difficult proposition. Also, there are serious ethical questions associated with this approach to the treatment of genetic disease because the therapy will affect not only the patient but his or her offspring as well. Some of these points will be discussed later.

At the present time, only somatic cell gene therapy is being pursued as a realistic possibility. This approach seeks to modify the genetic make-up of non-reproductive cells only. The basic approach currently is to choose a genetic disease characterized by a defective gene that causes an enzyme deficiency in the victim. The deficiency in turn causes the disruption of some essential metabolic process that is expressed as a disease syndrome. Medical researchers have now developed an understanding of the disease process in about 250 genetic diseases.(9)

One ideal gene-therapy technology would probably involve packaging the normal gene in some fashion and administering it to the patient by a single injection. The corrective gene would then find its way to target cells in the appropriate tissue - for example, to bone marrow cells in sickle-cell anemia or to muscle cells in Duchenne muscular dystrophy. Once inside the cell, the normal gene would become a permanent part of the genetic structure, direct the production of the needed metabolite, and all symptoms of the disease would disappear. The cure would be permanent.

Such technology is not currently available. Injecting DNA into the blood stream would be ineffective, either because the naked DNA would be destroyed by chemicals in the blood or by the patient's immune system. Nor is it possible to direct the cloned gene to the correct cells by injection. If incorporation of the DNA occurred at all in this

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(9) Nichols (1988), p. 17.

scenario, it would occur randomly throughout the body, the gene would become lodged in tissues where it was not needed, and reproductive cells might be also be affected.

For these and other reasons, medical researchers are directing their efforts toward techniques that involve the insertion of cloned genes into cells that have been removed from the body and, after treatment, are returned to the body. While the reason for this is pragmatic, the approach also has a built-in safety factor. If something goes obviously awry in the gene-transfer process, the attempt can be aborted with no harm to the patient. At the present time, only bone marrow cells and skin cells can be manipulated in this manner.

It is necessary also that the product of the normal gene, for example an essential enzyme, reach those parts of the body where it is needed. For some genetic diseases, it would be desirable for the gene product to reach the bloodstream, there to exert its beneficial effects. Because bone marrow produces the many types of blood cells which circulate throughout the body, and because bone marrow transplantation is an established medical technology, this will probably be the tissue chosen for the first trials in human gene therapy.

There are problems with bone marrow, however, and these have contributed to delays in the practical application of gene therapy. These problems derive from the fact that the blood-forming system in mammals is extremely complex. The cell type in bone marrow which forms the basis of the system is the stem cell, a primitive undifferentiated cell type which, as it matures through a series of steps, changes into the various component cells of the blood system. The stem cell will continue to divide indefinitely, in essence for a lifetime. In contrast, the cell types derived from the stem cells have finite life spans.

Therefore, the target cell for gene insertion is the stem cell, which produces a continuous supply of blood cells containing the inserted gene to cure the genetic disease. Insertion of the gene into the more mature cells in the bone marrow would not be effective because of their finite life spans. When these cells died, the inserted gene would be lost and the symptoms of the genetic disease would reappear.

Unfortunately, only about 0.01% of bone marrow cells are stem cells, and these are difficult to distinguish from the many other, more mature, bone marrow cells.(10) Thus, the gene transfer system which is chosen will have to be extremely efficient to ensure that a sufficient number of stem cells receive the normal gene.

There are additional problems. Blood cells go through many different states of development during the maturation process. It appears, from recent research, that genes are "switched" on and off within these maturing cells at the different stages. This characteristic of the maturation process may interfere with the expression of the "foreign" gene inserted to correct the genetic defect.

The insertion of a gene into the cells of mammalian tissue, or any other tissue, is a difficult proposition. Ideally, the medical scientist would like to replace the defective gene with its normal counterpart which would then function within the cell in the correct manner, producing the right amount of protein when switched on by the cell, and ceasing to produce when switched off. Science is still well short of that ideal, but techniques do exist for inserting foreign genes into target cells, although not yet into specific chromosomes. There is no technology available to inactivate a gene in, or remove it from, an animal, although this has been achieved with single-celled organisms such as bacteria and viruses. It has also been done with animal cells grown in artificial culture. The proposed gene-therapy techniques for humans are, therefore, "gene-addition" techniques rather than "gene-correction" techniques.

Two other objectives are important in this process, and have already been alluded to. One is to achieve appropriate expression of the inserted gene, that is, to make it produce the right amount of protein. The second is to ensure that the inserted gene does not harm the target cell or the patient.

There are three categories of techniques currently available for inserting foreign genes into mammalian cells. These are: (1) techniques using viruses, especially retroviruses; (2) chemical and physical techniques; and (3) fusion techniques. Of the three, the use of

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(10) Ibid., p. 103.

retroviruses, through the creation of a retroviral vector, is considered to be the most promising.(11) In the following paragraphs, the use of retroviruses will be the only technique discussed, although a number of others are being developed.

A. The Retroviral Vector

A virus, in simplified concept, consists of a nucleic acid core wrapped in a protective protein coat. The viral nucleic acid is analogous to a chromosome and may consist of either deoxyribonucleic acid, DNA, or the related ribonucleic acid, RNA. Often viewed as being on the borderline between a living and non-living entity, a virus essentially has a single function: to reproduce. To accomplish this very limited objective, a virus is obliged to infect a living plant or animal cell. Once inside the host cell, the virus becomes an intracellular parasite.

Again, in a very simplified concept, the virus infects by attaching itself to the wall of a living cell, and "injecting" its nucleic acid core into the cell. The viral nucleic acid, or genome, contains genes for taking over and re-ordering the activities of the host cell, including directing the production of molecular components for new virus particles. In many virus diseases, the disease cycle (at the cell level) ends with the release of hundreds of "daughter" virus particules and the death of the host cell. The daughter viruses then go on to infect other cells in the host organism.

Other viruses follow a different path of infection. These viruses may incorporate their nucleic acid into the host cell genome, and perhaps exert little effect on the survival of the host cell. The appearance and activity of the host cell, and of the host, may be profoundly affected, however.

Retroviruses comprise a unique group of viruses whose genetic material is RNA, rather than DNA. These viruses have the ability

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(11) Ibid., p. 108.

to infect a cell and then copy their RNA into DNA inside the host cell. This is accomplished through the activity of a retroviral enzyme known as reverse transcriptase. The DNA formed by this process is then incorporated into the genome of the host cell. In this state, the retrovirus is known as a provirus, the name indicating that it has the capability of changing, at some point, into an active virus. A feature of retroviruses that makes them attractive as delivery vehicles, or vectors, in gene therapy is that, in their natural state, they often carry foreign (non-viral) genes.

The conversion of a naturally-occurring retrovirus into a vector for the insertion of normal genes into mammalian cells is a difficult and complicated exercise. The retroviral vector must be able to infect the host cell, to copy its RNA into DNA, and to insert its genome into the host chromosome. The provirus made by the vector should be structured to direct the production of the desired human protein(s), but no viral proteins; that is, the provirus must not be allowed to direct the production of new virus particles.

The creation of a retroviral vector starts with the insertion of the relevant human gene(s) into the retroviral provirus in a host cell.(12) This is accomplished using recombinant DNA technology, commonly known as "gene splicing". The insertion of the human gene(s) is done in the provirus stage because it is easier to work with DNA than with the RNA that comprises the retroviral genome. In the same procedure, the provirus is further modified by removing from its genome the three genes that code for those viral proteins that are necessary to convert the provirus into an active retrovirus. The provirus retains the genetic elements needed to insert its DNA into the DNA of host cells, however.

Because the provirus has necessarily been modified in such a manner as to preclude its activity as an infectious virus, it is essentially "trapped" in the chromosome of its host cell. The second step in the creation of a gene-delivery system is the formation of a helper cell, also called a "packaging" cell. The helper cell will have the ability to

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(12) The retrovirus most often used is the Moloney murine (mouse) leukemia virus, or MoMLV. The host cells used to propagate, and modify, the vector provirus are mouse cells grown in artificial cell culture.

produce the viral proteins needed to package the recombinant provirus vector.

This essential step is accomplished by a "helper" virus imprisoned within the genome of the helper cell. The helper virus, like the vector provirus, is defective, but in a manner complementary to the provirus; that is, each of the two components of the gene-delivery system has abilities that the other lacks. Like the vector provirus, the helper virus has been modified by recombinant DNA techniques, within its host cell, and it lacks the ability to become encapsulated in viral proteins. However, it contains the genes necessary for the manufacture of those viral proteins, which are consequently present in the cytoplasm of the helper cell.

The third and final step in the creation of this gene-delivery system, the production of the actual retroviral vector, occurs within the helper cell. Copies of the vector provirus (which contain the human gene) are added to helper cell cultures under conditions that promote the uptake of DNA into the cells. In some of the cells in the culture, the vector provirus will become integrated into the helper cell genome. In the course of normal cell metabolism, the DNA (including the vector provirus) is copied into RNA.

Because the vector provirus contains the gene for "packaging" the retroviral RNA into the viral protein core and envelope, and because (as noted above) those proteins are present in the helper cell, the vector RNA is automatically encapsulated in the proteins and complete viral particles are created. These viral particles are then released from the helper cell by a process of "budding" from the helper cell membrane.

These viral particles, which in a sense are "hybrid" viruses, are infective, but they will only be able to infect a cell once; the hybrid viral genome lacks the genes for making viral proteins. Also, because the helper virus provided the protein for the viral envelope, the characteristics of the helper virus determine the types of host cells that the retroviral vector will be able to infect.

The last step in gene transfer involves exposing the target cells (for example, bone marrow cells from the patient) to the retroviral vector. This can be done by mixing the target cells with a solution of

helper cells that are producing the vector virus. Alternatively, the target cells can be bathed in fluid harvested from the helper cell culture. In either case, the retroviral vector binds to the cell membrane of the target cell, and the viral core is injected into the cell. There, the RNA undergoes reverse transcription to DNA and becomes integrated into the target cell genome. This gene-transfer process is called transduction.

B. Constraints and Potential Problems

Experimental work in many laboratories, using cell cultures, has shown that gene transfer using retroviral vectors is achievable, but the technique has not been perfected and there are certain problems. One is that the insertion of foreign gene(s) into the genome of a retrovirus often reduces the reproductive capability of that retrovirus. Another problem is that elements in the viral genome may interfere with the proper expression of the inserted human gene. If this should happen, the desired product of the gene, for example an essential enzyme, might not be produced in sufficient quantity, or at all, and the attempted therapy would have failed.

There are also problems involving interactions between retroviral vectors and transduced target cells. In studies with transduced bone marrow cells transplanted into experimental animals, the expression of the foreign genes sometimes appears to become shut off after a period of time. It is not known why this happens.

Two other concerns about the use of retroviruses have been alluded to above. One is that the retrovirus might become generally infectious and carry the foreign gene to non-target tissues in the patient's body. As has been explained above, both the original retrovirus and the helper retrovirus are genetically modified to render them defective and non-infective by themselves.

However, there is a theoretical possibility that the retroviral vector might exchange genetic material with the helper provirus, with another retrovirus resident in the helper cell genome, or with a retrovirus in the genome of the human target cells. Such an exchange of genetic material is common between microorganisms with similar genomes and

is called recombination. Fortunately, scientists have a number of ways of reducing the probability that such recombination will occur in gene therapy.

A second concern is that the retroviral vector, after its DNA is inserted into the target cell genome, might activate an entity known as a proto-oncogene. Proto-oncogenes are associated with cancer because they can be activated, or altered, to turn into oncogenes, which are powerful inducers of unregulated cell growth. Experiments with laboratory animals have shown that the insertion of a retroviral provirus into a chromosome next to a proto-oncogene can provide the necessary stimulus for a cancerous transformation.

The human genome contains only a very small number of proto-oncogenes compared to its total number of genes. There is, therefore, a low probability that the retroviral provirus will be inserted into a chromosome next to a proto-oncogene. For this, and other reasons, there is very little chance that gene therapy will cause the formation of a human cancer.

DIAGNOSIS OF GENETIC DISEASE: PRENATAL AND CARRIER SCREENING

It is obvious that a disease must be accurately diagnosed so that the appropriate treatment may be prescribed. For many genetic diseases, notably those that affect newborn children, the timing of the diagnosis can be very critical. With phenylketonuria (PKU), for example, afflicted infants cannot metabolize the amino acid phenylalanine. If not placed on a phenylalaline-restricted diet, the children will suffer severe mental retardation as the chemical builds up in the body and causes damage to brain cells.

A similar situation exists with congenital hypothyroidism. The afflicted infants are unable to produce thyroid hormone. Unless the hormone is administered quickly and regularly, the babies will develop the symptoms of cretinism, a syndrome involving irreversible mental retardation, deafness, and abnormal skeletal growth.

For some genetic diseases, such as a predisposition to late-life diabetes or heart disease, early diagnosis can have very beneficial effects through modification of life-style and diet. The individual will remain susceptible to the disease, but he/she will have at least delayed the onset of the condition and lived a fuller life in the interim.

For many other inherited conditions, however, there is no effective treatment. Here, in a sense, medical science only accords formal identification to a situation it is powerless to modify. Such identification, however, has led to use of sophisticated diagnostic procedures. One is prenatal screening by one of a number of techniques to determine if the fetus is carrying a genetic disease or is otherwise abnormal. The second is carrier screening to determine if an individual carries in his/her genome a recessive gene for a specific disease. Such knowledge could have an obvious effect on an individual's decisions about becoming a parent.

A carrier, strictly defined, usually does not develop symptoms of the genetic disease, because the product of the single normal gene is usually sufficient for a healthy life. For some dominant genetic diseases, however, symptoms will not develop until fairly late in life, long after the individual has reached reproductive maturity. In this sense the person is a carrier because he or she may unknowingly transmit the gene(s) to the next generation. The classic case is that of Huntington chorea, a progressive and fatal neurological affliction whose average age of onset is 38 years.

A. Prenatal Diagnosis and Screening

There are six main techniques of prenatal diagnosis to identify genetic diseases and other fetal abnormalities. Each of the techniques will be described briefly.

(i) Amniocentesis: Performed at 15-16 weeks of pregnancy, this is the most widely established technique. It involves the insertion of a needle through the mother's abdominal wall to withdraw a small amount

of amniotic fluid surrounding the fetus. This fluid contains cells from the fetus which can then be analyzed to detect major chromosome abnormalities and some biochemical defects. The technique has an approximate one in 240 risk of inducing a miscarriage.(13)

(ii) Fetoscopy and Fetal Blood Sampling: Fetoscopy is a technique which uses a fiberoptic endoscope, a fine fiberoptic tube with a lens on the end. The endoscope is inserted into the womb so that the physician is able to observe the fetus directly. The technique is usually performed at 17-18 weeks or more of gestation. Vizualization permits the detection of external malformations of the fetus; these may not be genetic in origin. In 1974, fetoscopy was adapted to sample fetal blood, allowing physicians to detect such blood diseases as thalassemias and sickle cell anemia. Other genetic disorders affecting blood and blood products can also be diagnosed. Recently, it has become possible to sample fetal blood without fetoscopy, using a fine needle guided by ultrasonograpny (see below). The risk of miscarriage with fetoscopy is about 4%,(14) with fetal blood sampling, about 2%;(15) both rates are significantly higher than for amniocentesis.

(iii) Ultrasonography: Recent advances in ultrasound technology have permitted the production of high-resolution images of the fetus. These images can be used to detect skeletal disorders, central nervous system defects such as anencephaly (a condition in which part of the brain is missing or much reduced in size) and hydrocephalus (accumulation of fluid on the brain), kidney abnormalities and urinary tract obstructions. In combination with other techniques, ultrasonography can be used to assess the structure and function of the fetal heart. The technique is also useful in helping to guide biopsy needles to obtain fetal

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(13) M. d'A. Crawfurd, "Prenatal Diagnosis of Common Genetic Disorders," British Medical Journal, Vol. 297, 20-27 August 1988, p. 504.

(14) Ibid.

(15) Nichols (1988), p. 44.

blood or liver tissue. Ultrasonography is probably harmless to mother and fetus but many abnormalities cannot be detected until 18-20 weeks' gestation or later.(16)

(iv) Chorionic Villus Sampling (CVS): The chorionic villi are fingerlike projections of the membrane surrounding the embryo early in pregnancy. CVS can be used in the ninth through eleventh weeks of gestation to obtain cells for diagnosis. This confers an advantage over amniocentesis and fetal blood sampling, neither of which usually will give results until the second trimester, at which time a therapeutic abortion of a seriously defective fetus is much more difficult for the mother than one in the first nine weeks. The tissue may be harvested by one of two routes: by a catheter inserted through the vagina and cervix into the uterus, or by needle through the abdominal wall, as in amniocentesis. The technique has an approximate 2% risk of causing miscarriage.(17)

(v) Maternal Serum Alpha-Fetoprotein (MSAFP) Sampling: AFP is a protein found in the blood serum of all pregnant women and also in the amniotic fluid. The test is relatively simple - test kits have been available to medical laboratories since 1983 - and entails no risk for the mother or fetus. The test is based on the fact that a woman carrying a fetus with spina bifida, a serious defect in which the spinal column fails to close during development, has a higher-than-normal MSAFP level. Also, large screening programs have shown that a woman carrying a fetus with Down syndrome will have a lower-than-normal MSAFP level. The test is useful for screening purposes but the results indicate only that the mother has a higher probability of carrying a defective fetus, as described. A positive MSAFP test is not conclusive by itself. For example, surveys have shown that of 50 women who have a high MSAFP level, only two will be carrying a fetus with actual structural defects, and another 28 fetuses will appear normal but may be at increased risk of future problems. Similarly, a low MSAFP level indicates that there is a higher probability that the fetus has

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(16) Crawfurd (1988), p. 504.

(17) Nichols (1988), p. 45.

Down syndrome; in a 34 year old woman, the probability increases from about one in 350 to greater than one in 150.(18)

(vi) Recombinant DNA Techniques: The harvesting of fetal cells for diagnostic analysis by biochemical techniques has a major disadvantage in that the specific chemical deficiency must be known before the test can be done. Also, the right kind of fetal cell must be obtained to carry out a specific test: cells gathered through amniocentesis, CVS, or fetal blood sampling cannot be used to test for a disease like phenylketonuria (PKU) which is caused by a malfunction in liver cells, since these cells cannot be obtained in this way.

This is not a problem with the new recombinant DNA (rDNA) techniques, which focus directly on the structure of the genome. The gene which causes PKU is present in all cells even though it is active almost exclusively in the liver. A test for the gene itself, rather than the product of the gene, is a major advance in the diagnosis of genetic disease.

The rDNA techniques use restriction enzymes, the restriction endonucleases described earlier in the discussion of molecular genetics and sequencing of nucleotides. Restriction enzymes cut the DNA at specific recognition sites, that is, at the point of specific sequences of nucleotides. In their DNA, different individuals possess variations in restriction enzyme recognition sites. For example, the same enzyme that may cut a piece of DNA from one person into three fragments, may cut the corresponding piece of DNA from another person into only two fragments. This means that the second person is missing a recognition site, something that can happen with a change in a single nucleotide or base-pair.

These variations in DNA between individuals are called restriction fragment length polymorphisms or RFLPs. The actual variations in RFLPs rarely cause genetic diseases themselves, but it has been found that in some cases they can serve as indirect markers for certain genetic diseases. The closer an RFLP is to a defective gene on the chromosome, the greater the chance that it will be inherited with that gene. A "catalogue"

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(18) Ibid., p. 54-55.

of specific RFLPs on various chromosomes in the human genome is a valuable asset for studying the inheritance of human traits, including genes for genetic diseases.

The development of an rDNA test for a specific genetic disease involves studying DNA specimens from healthy and affected members of a family with that genetic disease. The objective of the study is to find a DNA pattern, expressed as RFLPs, that is linked to the disease. This process is called linkage analysis. If such a pattern is found, and can be verified in an appropriate number of people, a standard diagnostic or screening test may be developed.

This technique is successful with a number of diseases. As suggested, the accuracy of the test is a function of the closeness on the chromosome of the RFLP to the defective gene. For sickle cell anemia, the test is perfectly accurate because the RFLP appears to coincide with the defective gene itself. The linkage analysis test for the Duchenne muscular dystrophy gene has an accuracy level between 90 and 99%.(19) The accuracy of the test for the Huntington disease gene appears to be about 99%.(20)

There is an alternative technique for detecting point mutations in DNA. This technique employs short pieces of synthetic DNA called oligonucleotides which will, under test conditions, bind to matching sequences of human DNA. When constructed with radiolabelled chemical constituents, these can be used as "probes" to distinguish between normal and defective genes. Probes have also been constructed to identify RFLPs closely linked to disease genes.

B. Carrier Screening

By use of various techniques it is possible to detect carriers of defective genes, even before those carriers have had children. In the past, a carrier could not be identified until a defective child had

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(19) Ibid., p. 49.

(20) Lawrence Surtees, "Doctors Using New Genes Probe in Huntington's Disease Program," The Globe and Mail, 29 August 1988.

been produced. The carrier-screening tests are mainly applicable to diseases caused by autosomal recessive and X-linked genes since such genes can be carried by persons who will not actually develop the disease. Carrier detection and screening can have a profound effect on the numbers of children born with genetic diseases.

For example, in 1970, before a carrier-screening program was initiated, 50-100 babies with Tay-Sachs disease were born each year in the United States. Today, the total has dropped to 10 or less.(21) Tne screeningprogram has given couples found to be at high risk the option of having only normal children, and from that point of view the program nas been a success. There are a number of aspects of carrier screening tnat may be difficult for some individuals and groups to accept, however, and these will be discussed below.

SOCIAL AND ETHICAL ISSUES

A. Somatic Cell Versus Germ Line Gene Therapy

The discussion of the social and ethical aspects of gene therapy must at once be divided, like the technical discussion, into two distinct categories, reflecting the intrinsic difference between somatic cell and germ cell therapies. It is perhaps a fine philosopnical and biological point, but the two types of tissue are fundamentally different, not only from a functional standpoint, but from an evolutionary and social perspective as well.

> Biologists have often emphasized the fact that in general the germ cells form an exceedingly independent tissue; the rest of the body, the "soma", is, in this point of view, merely a temporary structure shielding and conserving the potentially immortal germplasm.(22)

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(21) Nichols (1988), p. 56.

(22) Alfred Sherwood Romer, quoted in, David Suzuki and Peter Knudtson, Genethics: The Ethics of Engineering Life, Stoddart Publisning Company, 1988, p. 181.

The somatic cells of the body, although directed in their activities by the DNA in their chromosomes, and bearing the cumulative inheritance of millions of years of evolution, are essentially transitory. Nothing that happens to these cells and tissues can have any qualitative effect on the composition of the DNA of future generations.

Medical technology in this latter half of the twentieth century has made incredible strides in the treatment of disease. Among the most startling achievements are organ and tissue transplantation, perhaps the most dramatic and revolutionary of which is heart transplantation to cure terminal cardiac disease. Although there are some groups and individuals who may object to these procedures, by and large the general public now accepts organ transplantation as a standard medical practice.

It is in this context that somatic cell gene therapy must be considered. The transplantation of a gene into a somatic cell is, at a molecular level, analogous to organ transplantation, which also involves inserting into the patient cells containing "foreign" DNA. Somatic cell therapy exerts no effect on genes in the germ cells.

> Gene therapy of this type is therefore conceptually no different from any therapy in medicine that attempts to improve the health of a sick patient. The only difference is that DNA, rather than other biologicals, drugs or surgery is used as the therapeutic modality ... gene therapy for diseased tissues is not different from any other therapy. No change in the genes of the reproductive organs is attempted.(23)

In contrast, germ cell gene therapy (if it were possible) would be a radical departure from accepted medical practice. This procedure would seek to insert a gene into the genome of the fertilized egg to correct a specific genetic defect. That corrective gene would then be incorporated into every cell of the developing embryo and fetus, and ultimately be transmitted to the offspring of the individual. This intervention, which has a measurable impact not only on the patient but on succeeding generations, has no precedent in medical practice.

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(23) Arno G. Motulsky, "Impact of Genetic Manipulation on Society and Medicine," Science, Vol. 219, 14 January 1983, p. 138.

An important tenet in medicine is that of "informed consent". Where someone is incapable, for whatever reason, of giving consent, a parent or guardian is typically granted that power and responsibility. The extension of that power to include control over the unborn fetus is currently controversial because of the unsettled issue of "rights of the unborn". Medical intervention that will exert effects on future generations will inevitably be controversial.

If germ line gene therapy could be carried out with complete effectiveness and absence of risk, there would probably be little concern (or at least much less concern) about it from an ethical point of view. Whatever one's qualms or feelings about medical intervention in this area, it is hard to imagine that one would argue against a technology that would eliminate the genes for Huntington chorea or Lesch-Nyhan syndrome from the human gene pool.

The controversy over germ line gene therapy stems more from two other concerns. There is the obvious fear that an imperfect intervention might result in heritable deleterious changes that would affect future generations and would, moreover, become part of the general human genetic inheritance. This would be, in a very small way, an unwanted alteration of the human gene pool.

The second concern is that if science developed a way to treat human disease by altering certain genes in the germ line, there might be a desire to extend this application to "improving" the human gene pool in a eugenic sense. If this idea took hold, subjective decisions would be made about what characteristics were desirable and what were not, with the latter being targeted for elimination. For some observers, this is a real possibility and one that must be guarded against. For others, the proposition is fanciful at best.

In addition to the complex ethical and legal problems associated with germ line gene therapy, there remain immense technical difficulties which are much greater than those for somatic cell therapy. Although germ line genetic intervention has been achieved with animals, such as laboratory rodents and some agricultural species, the nature of that intervention is very different from that envisaged for human patients.

There are now many examples of transgenic animals which have been "created" by inserting a gene, or genes, from a different species into the genome of a fertilized egg.(24) The first and best-known examples are the "super-mice" which were created by inserting copies of the gene for rat growth hormone into mouse embryos. Some of the progeny mice grew to much larger size than their untreated litter-mates (hence the name super-mice), and subsequent generations also carried and expressed the inserted genetic trait.(25)

Even at the level of experimental and farm animals, however, germ line intervention is controversial for ethical and moral reasons. On the practical side, also, there are extreme difficulties. To create a transgenic animal, the scientist (in the most common technique) uses a microscopic pipette to inject copies of the desired gene directly into the pronucleus of the fertilized egg.(26) The major problem is that most of these fertilized eggs will not survive the trauma of the treatment.

There are two other major problems. One is that there is no way, currently, to diagnose genetic diseases in the fertilized egg, the stage at which treatment would have to be implemented. As noted earlier, the most recent technology facilitates diagnosis of a genetic defect toward the end of the first trimester of pregnancy. (In very rare cases it is possible that two individuals homozygous for the same autosomal gene could mate and, in this case, the fetus would also be homozygous for the same disease gene and be known to have the disease.)

The third problem is that there is also no way, with current technology, to control where the corrective gene will be inserted into the

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(24) Ian Wilmut, John Clark and Paul Simons, "A Revolution in Animal Breeding," New Scientist, 7 July 1988, pp. 56-59.

(25) R.D. Palmiter et al, "Dramatic Growth of Mice that Develop from Eggs Microinjected with Metallothionein-Growth Hormone Fusion Genes," Nature, Vol. 300, p. 611-615, 1982.

(26) A pronucleus is the mature haploid nucleus of either the sperm or the egg which contributes to the formation of the fertilized zygote nucleus, after entry of the sperm into the egg.

embryo's genome, although some progress is being made in this area.(27) Arguably, it is acceptable to use a "shotgun" approach to germ line intervention with animal species, but such an approach is not acceptable for human patients. The inability to direct the insertion of the desired gene precisely also applies to somatic cell gene therapy, but, there, only a single target tissue would be affected.

The potential consequences of random insertion of a foreign gene into every body cell are very problematic. An inserted gene, as was noted earlier, may exert effects on the expression of other genes in a chromosome. For example, the metabolic activities that take place in muscle cells are quite different from those in pancreatic cells, altnougn each type of cell has the same genetic make-up. Obviously, the same genes are not active in each type of tissue. A foreign gene may, therefore, exert unpredictable and varying effects, depending on the type of tissue into which it is inserted.

In the medical community, there appears to be almost unanimous agreement that germ line gene therapy should not be attempted with humans, at least not yet. The Medical Research Council of Canada (MRC), the federal government's principal granting agency for medical research, through its Standing Committee on Ethics in Experimentation, has stated the following policy position:

> The most likely and practical applications of genetic engineering in human beings ... closely resemble well-accepted medical interventions and provide promising approaches to correct certain well-understood, but currently untreatable, genetic deficiency diseases caused by a single defective or missing protein...
>
> Another technique, as yet at an early stage of testing, is germ-line genetic engineering. This changes not only the genetic character of the individual but of the offspring as well, because it affects the sperm and ova, the germ cells. The ethical implications in this type of transfer cause grave concern. There are profound difficulties in estimating risk and benefit.

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(27) Jean L. Marx, "Gene Transfer is Coming on Target," Science, Vol. 242, 14 October 1988, pp. 191-192.

> In the present state of knowledge, the Committee advocates consideration of human genetic engineering only where there is no reason to believe that the genetic alterations will be inherited, and only when long-term follow-up is an element of the research proposal.(28)

If the concept of genetic intervention can be accepted, if only at the somatic cell level, then the important considerations are the criteria for selecting patients for treatment. The principal concern must be the welfare of the patient; if effective treatment already exists for his disease, a patient should not be considered at this time as a candidate for gene therapy. Gene therapy is a new treatment and great caution must be exercised in the initial trials.(29) With today's technology, only single-gene disorders are being considered for treatment, with the inserted gene supplying a metabolic product that will correct the deficiency disorder.

Six criteria have been elaborated for somatic cell gene therapy, to be based on clinical and laboratory evidence developed for each candidate:

(1) the disease is life-threatening and incurable without gene therapy;

(2) the organ, tissue, and cell types affected by the disease have been identified;

(3) the normal counterpart of the defective gene has been isolated and cloned;

(4) the normal gene can be introduced into a substantial fraction of cells from the affected tissue; or introduction of the gene into an available target tissue,

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(28) Medical Research Council of Canada, Guidelines on Research Involving Human Subjects 1987, Minister of Supply and Services Canada, Ottawa, p. 17.

(29) In July 1980, two unauthorized attempts were made to treat thalassemia victims through somatic cell gene therapy. The physician was Dr. Martin Cline of the University of California at Los Angeles; the patients were in Italy and Israel. Neither attempt was successful and Dr. Cline was both reprimanded and punished (by cancellation of research grants) for his efforts. Neither patient appears to have been injured by the treatment.

such as bone marrow, will somehow alter the disease process in the tissue affected by the disease;

(5) the gene can be expressed adequately; that is, it will direct the production of enough normal protein to make a difference in the patient's condition;

(6) techniques are available to verify the safety of the procedure.(30)

The first authorized efforts at gene therapy, as discussed earlier, will probably be made with a disease which can be treated through insertion of a corrective gene into bone marrow, a tissue which can be treated outside the body. Nichols states that two rare genetic diseases appear to satisfy the six criteria. These are ADA deficiency and PNP deficiency (described earlier in the section on genetic diseases), each of which affects an individual's immune system.(31) The insertion of the normal gene for each disease into bone marrow should, in theory at least, result in the development of bone marrow cells with the capacity to produce the missing enzymes, adenosine deaminase (ADA) and purine nucleoside phophorylase (PNP).

B. Prenatal Screening

There are some issues associated with prenatal diagnosis and screening which are of legitimate concern and which may also be controversial. The most obvious of these is the use of therapeutic abortion where the fetus is found to be afflicted with a genetic disease or some other abnormality. The abortion issue, with its many facets, is beyond the scope of this paper. However, it is pertinent to describe some of the situations that can arise in the present context.

Of the more than 200 genetic diseases that may now be diagnosed in the womb, all are incurable, some are treatable, and some are invariably fatal. For an incurable and untreatable disease like Tay-Sachs, for example, in which the quality of life begins to deteriorate seriously after four or five months, and which results in death by four years of age,

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(30) Nichols (1988), p. 18.

(31) Ibid., p. 39.

the arguments in favour of therapeutic abortion may be quite strong. For a disease like Down syndrome, however, the situation is much less definitive because the victim may often enjoy a good quality of life well into adulthood, although mental retardation and other problems are to be expected. Similarly, the situation involving a disease like cystic fibrosis will also be very difficult. The victim will have a difficult and truncated life, but that short lifetime will not be without quality.

Another situation arises with a disease like Huntington chorea, which does not usually develop until the victim reaches his/her late thirties. For more than half a typical lifespan, the victim can live a normal life. When the disease does strike, the physical and mental manifestations are terrible and the effect on family and friends is immense. For a parent whose child might be prenatally diagnosed as naving Huntington (or a similarly-devastating disease) the moral dilemma will be profound, perhaps insoluble. The situation is even more complicated with Huntington because the disease is caused by an autosomal dominant gene, meaning that one of the parents, the gene carrier, must also develop the disease.

There is no question that many parents, upon learning that a fetus has an incurable genetic disease will choose to abort, rather than face the pain and trauma of dealing with the affected child. The frequency of abortion will probably be more or less proportionate to the seriousness of the disease.

Two other aspects of the abortion issue must be mentioned, nowever. First, there is the obvious fact that, if an effective and safe therapy is developed for a genetic disease, prospective parents will be much less likely to opt for abortion than if no therapy is available, whatever the nature of the disease.

The second aspect will tend to reduce the number of abortions also, below the number that would occur in the absence of prenatal testing. Many high-risk couples - that is, those who have already given birth to an affected child, or who have been identified as carriers of defective genes - stop having children and subsequent pregnancies are usually unintentional. In a study of beta thalassemia, a condition

producing severe anemia, it was found that 70% of these accidental pregnancies were ended by abortion. When prenatal diagnosis for beta thalassemia was developed, the rate of abortion dropped to 30%. The additional 40% of fetuses aborted prior to the use of prenatal diagnosis would presumably have developed into normal babies. Similar results have been reported for many other genetic disorders.(32)

C. Carrier Screening

The matter of carrier identification raises some difficult issues. First is the fact that the tests are not always completely accurate and there exists the possibility of false positives. Second, with some diseases, and Huntington is an example, some carriers of the gene will be unable to cope with the knowledge that they will develop the disease. Third, and this applies to a number of diseases, there is the question of the use, and possible misuse, of diagnostic information.

The less-than-perfect accuracy of a diagnostic test, while not restricted to genetic diseases, is a serious problem. If a disease is life-threatening, or otherwise seriously traumatic in its manifestations, potential victims may want to know so that they can make appropriate decisions about their lives. If one carries a gene for such a disease, there is an obvious concern about reproduction. If one already has a spouse and children, certain financial and career decisions may have to be made, to deal with the reality of premature incapacitation or early death. The accuracy of the diagnostic test is an important consideration in such critical situations.

The ability to predict that one will develop a terminal disease has a dark side that merits serious consideration before such a test is made freely available. Again, the worst-case scenario has been developed for Huntington:

> Predictive testing for Huntington chorea promises great benefits to those who prove not to be carriers, but it has been suggested that those shown to be carriers

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(32) Ibid., p. 42.

> will be deprived of hope and will be at greatly increased risk of depression and suicide.(33)

There is a delicate balance here between the need to know, the wish to make rational decisions about one's life and activities, and the basic human response to a virtual death sentence. Each individual at risk from a disease like Huntington should be allowed to make his or her own choice in the matter of predictive testing.

A national genetic-screening program is being established in Canada for Huntington chorea. The program will be directed from the University of British Columbia in Vancouver. The program will offer people with a documented family history of Huntington a test to determine their risk of developing the disease. The program has adopted strict criteria to ensure that each person tested is prepared psychologically, through counselling, to accept a possible positive finding.(34)

The possible misuse of information from predictive testing and carrier screening has been widely discussed. The concern about misuse is predicated on the fact that test results will be of interest to others besides the person at risk. In some cases, this could lead to conflict. The conflict could arise in the family situation, in relations with real or potential employers, and with insurance companies.

There are a variety of ways in which family conflict might arise. The spouse of a person at risk may wish to know if he or she is a carrier, but the person at risk might not. Children may not want a parent to be tested if the results might mean that they themselves are at increased risk. Some predictive testing (as is done through linkage analysis using RFLPs) requires that many family members participate in the screening program: some family members may not wish to participate and may not wish to have the information, thus compromising the accuracy of the test.

In the workplace, suggestions that carrier screening be implemented is controversial. Initially, researchers believed that the

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(33) D.I.O. Crawfurd and R. Harris, "Ethics of Predictive Testing for Huntington Chorea: the Need for More Information," British Medical Journal, Vol. 293, 26 July 1986, p. 250.

(34) Surtees (1988).

identification of individuals who are more susceptible to specific workplace toxins than their colleagues, and their exclusion from these hazardous occupations, would be a positive development in preventive occupational toxicology. In fact, the proposal to develop such tests was viewed by critics as paternalistic and discriminatory.(35) This would certainly be correct if the screening test were inaccurate, inappropriate or unfair. It would also be unacceptable to use genetic screening, and exclusion of affected individuals from employment, as a substitute for achievable standards of industrial hygiene.

If it can be shown, however, that certain genetic traits are in fact indisputably associated with sensitivity to specific levels of certain chemicals, dusts, or other workplace contaminants which are demonstrably harmless to workers without those genetic traits, and those various contaminants cannot practicably be reduced below the specific danger levels, then it becomes difficult to argue that sensitive individuals should not be excluded from those particular environments. This would not be employment discrimination; rather, it would be the prudent application of preventive medicine:

> If genetic tests are eventually devised that can reliably identify workers who are genetically most at risk for developing serous occupational illnesses and if these tests are used in an ethical manner, everyone stands to benefit. Genetic screening programs could prove economically beneficial not only to employers and employees but also to life insurance companies, medical insurance companies, government health plans and other groups or plans that try to anticipate the health risks of working men and women.(36)

The impact of carrier-screening information on decisions made by life insurance companies has been discussed by many writers in this field. Candidates for life insurance are subject to medical evaluation to prevent the issuing of policies to individuals who are poor health risks. The question that might be asked is whether insurance companies will

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(35) Gina Kolata, "Genetic Screening Raises Questions for Employers and Insurers," Science, Vol. 232, 18 April 1986, pp. 317-318.

(36) Suzuki and Knudtson (1988), pp. 169-170.

require genetic tests for people at risk for diseases such as Huntington, as more such tests become available and their accuracy improves. If so, will the information obtained remain confidential or become available to other interested parties, such as potential employers?

As this debate develops, it will be noted that genetic diseases are probably not increasing in frequency in the population, and may even be decreasing as some high-risk couples opt to remain childless. Therefore, the actuarial tables on which life insurance companies base their business decisions must already be taking into account the fact tnat a proportion of policy-holders will develop life-shortening genetic diseases. If the industry decides to eschew genetic testing, the situation will remain as it exists.

Gene therapy is sometimes criticized on the grounds tnat it may eventually be adapted to alter the genetic traits of "normal" individuals as part of some eugenic program to produce "better" people. Tnis argument, popular with some groups, obscures the fact that the tnerapy is being developed as a means of dealing with devastating inherited diseases for which there is no cure, often no treatment, and which are frequently fatal in early life. The concern that a technology might be misused, or adapted to achieve undesirable objectives, is quite valid and must be incorporated into the governmental decision-making process. It is not, by itself, an acceptable argument for banning a technology or thwarting research activities.

The fact that genetic diseases are usually individually rare is not a persuasive argument against the development of a therapy. Tne initial cost of gene therapy will be very high. However, taken as a group, genetic diseases affect a large number of people. As tne technology of gene transfer and control progresses, through continued research, more of these diseases should become treatable. It is possible that the cost of individual treatments will decrease as the technology matures and improves. Also, the cost of therapy must be weighed against the present cost of treatment of victims of genetic disease. Some of these costs are also very high and may have to be borne for the lifespan of the patient.

Finally, the knowledge gained through the various research activities associated with the development of gene therapy is of enormous value by itself, in that it increases our understanding of genetics, developmental biology, and the mechanisms by which the genes exert their control over life processes. At least some of the new knowledge gained will be used to produce better standards of health care or to benefit society in other ways.

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GLOSSARY OF TERMS

ALPHA-FETOPROTEIN: A protein present in the blood of the fetus, infant, and the normal pregnant woman. Quantification of this protein aids in the diagnosis of spina bifida and Down syndrome.

AMINO ACID: The chemical building blocks of protein. Twenty different amino acids may be found in the proteins of humans.

AMNIOCENTESIS: A prenatal diagnostic procedure in which a needle is inserted through the abdominal wall of the pregnant woman to withdraw fluid from the amniotic sac (the fluid-filled sac surrounding the fetus). Fetal cells in the fluid can be analyzed for chromosomal abnormalities and genetic diseases. Alpha-fetoprotein also can be measured in the fluid.

AUTOSOME: Any of the chromosomes except the sex chromosomes (the X and Y chromosomes). Humans have 22 pairs of autosomes.

BASE: One of four specific chemical molecules in DNA - adenine, cytosine, guanine and thymine - the sequence of which contributes the informational content to nucleotide building blocks.

BASE PAIR: The bases form pairs on each strand of the DNA molecule. Adenine always pairs with thymine, and guanine with cytosine. This specific pairing forms the basis of DNA replication and information-transmitting capability.

BONE MARROW: The soft, pulpy material in the cavities of bones. The marrow contains the stem cells that give rise to all of the blood cells, including elements of the immune system.

CARRIER: A person who carries one copy of a gene associated with a recessive genetic disease, and one copy of its normal counterpart. The carrier usually does not show symptoms of the disease.

CHORIONIC VILLUS SAMPLING (CVS): A prenatal diagnostic procedure in which samples of the chorionic villi (fingerlike projections of the membrane surrounding the embryo early in pregnancy) are collected. The villi have the same genetic makeup as the fetus. Analysis of the cells by various methods can lead to identification of genetic diseases.

CHROMOSOME: A structure in the nucleus of the cell that contains the hereditary information encoded in the genes which are composed of DNA.

CLONING: Applied to genes, it refers to the use of bacteria or viruses to produce millions of copies of a specific sequence of DNA.

CYTOPLASM: All of the contents of a cell except the nucleus.

DNA: Abbreviation for deoxyribonucleic acid, the molecule that contains the hereditary information in all living creatures, except for some viruses which contain ribonucleic acid or RNA.

ENZYME: A protein molecule that catalyzes chemical reactions in the living cell.

EXPRESSION: The activation of information in a gene.

FETOSCOPY: A procedure, utilizing a fiberoptic instrument, to view the fetus in the womb.

GENE: A sequence of DNA containing information for the synthesis of a single polypeptide or protein molecule. In classical genetics, a gene was defined as the fundamental unit of heredity that carries a single trait.

GENE SPLICING: The insertion of a sequence of DNA into a second DNA molecule in a "test tube".

GENOME: The total information contained in the genes of an organism.

HELPER CELL: Also called a "packaging cell", it is a cell that has been altered genetically to allow the production of a disabled virus capable of infecting target cells to achieve the insertion of a foreign gene in gene therapy.

HELPER VIRUS: A defective, or disabled, retrovirus used to assist the retroviral vector in inserting a foreign gene into the genome of a target cell.

HETEROZYGOUS: Having two different forms of a given gene on the paired chromosomes of a cell; for example, a cell may have one normal copy of a gene and one defective copy of the same gene.

HOMOZYGOUS: Having two identical copies of a given gene on the paired chromosomes of a cell.

LINKAGE ANALYSIS. The search for traits that may be inherited together, probably because their respective genes are close to one another on a chromosome.

MONOGENIC DISORDER: A disease caused by a defect in a single gene. Almost 4,000 monogenic disorders have been identified.

MUTATION: A change in the structure of DNA that alters the information it encodes.

NUCLEOTIDE: One of the building blocks of nucleic acids, DNA and RNA. Chemically, a nucleotide has three components: a base (in DNA, one of

adenine, thymine, cytosine or guanine; in RNA, uracil replaces thymine), a sugar molecule, and a phosphate group.

NUCLEUS: The structure in a cell containing the chromosomes.

ONCOGENE: A gene that is associated with the process of changing a normal cell into a cancerous cell. (see proto-oncogene)

PRENATAL DIAGNOSIS: The diagnosis of genetic diseases or other disorders in a developing fetus.

PROBE: A short piece of DNA or RNA of known structure or function which has been "tagged" with a tracer (such as a radioactive isotope) and which is used to locate and identify a specific gene or sequence on a chromosome.

PROTO-ONCOGENE: A gene capable of turning into an oncogene but which, under most conditions, participates in normal cell functions.

PROVIRUS: A copy of the genetic information of a retrovirus that is integrated into the DNA of an infected cell.

RECOMBINANT DNA: A DNA molecule assembled in a test tube using segments of DNA from two different sources. The segments are obtained by cutting a chromosome with restriction enzymes and "splicing" them together using an enzyme called DNA ligase.

RESTRICTION ENZYME: An enzyme produced by a bacterium which recognizes and cuts a specific nucleotide sequence, or "recognition site", of DNA. The enzyme has a protective function in the bacterial cell. The restriction endonuclease enzymes have been essential to the development of recombinant DNA technology. Almost 600 different enzymes are now known.

RESTRICTION FRAGMENT LENGTH POLYMORPHISM: An RFLP is the variation in the length of pieces of DNA obtained when two similar DNA molecules are cut by a restriction enzyme. This variation results from a mutation that has increased or decreased the number of recognition sites in one of the pieces of DNA. RFLPs are used in linkage analysis studies in carrier screening for genetic disease.

RETROVIRUS: One of a class of viruses that has RNA, rather than DNA, as the hereditary material. These viruses have the ability to copy their RNA into DNA inside the host cell and incorporate that DNA into the host genome as a provirus. For this reason, retroviruses have potentially important application as vectors in gene therapy.

RNA: The abbreviation for ribonucleic acid. RNA is the hereditary material in some viruses. In most living cells, RNA converts the information contained in DNA into protein or polypeptide synthesis in the cytoplasm of the cell.

SOMATIC: A term which refers to all the body tissues other than the reproductive, or germinal, tissues.

STEM CELL: A cell, found in the bone marrow, which gives rise to the entire spectrum of blood-forming cells. Stem cells can replicate themselves to give rise to more stem cells, or can differentiate into cell lines that form the red and white blood cells.

TARGET CELLS: A cell type which, in somatic cell gene therapy, is removed from a patient, has a corrective gene inserted into its genome, and is then returned to the patient, carrying the normal gene.

TRANSCRIPTION: The copying of a key set of nucleotides in the gene from DNA into a single-stranded molecule of "messenger RNA". This is a key step in the expression of a gene.

TRANSDUCTION: The transfer of foreign DNA into a cell using a virus as the vector.

TRANSFECTION: The transfer of foreign DNA into a cell by chemical or physical methods.

TRANSLATION: The process of decoding the information in a molecule of messenger RNA and using it to direct the construction of protein or polypeptide molecules specified in the messenger RNA.

ULTRASOUND: The use of high-frequency sound waves to produce body images. The technique is used in prenatal diagnosis.

VECTOR VIRUS: A virus that has been altered with recombinant DNA technology to carry foreign DNA (for example, a normal human gene) into the genome of a target cell.